REMARKS

Applicants representative would like to Examiner Hayes for his time with the very helpful interview of April 15, 2003. The amendments above and following remarks are submitted in an effort to address issues remaining in the application and facilitate the examination of the application.

Recitation of specific SEQ ID NOS

During the Interview, the Examiner suggested that the following features be defined in the claims:

1) The Examiner suggested that specific primers listed in the specification be incorporated into the claims. As suggested by the Examiner, the claims have been amended to incorporate the specific primers listed in the specification. It is Applicants' position that while the claims recite the primers consisting essentially of the indicated SEQ ID NOS, it will be readily appreciated by those skilled in the art that minor modifications of primer sequences can be made, for example, the insertion or deletion or mutation of one or a few bases, while maintaining the utility and function of the primer sequences.

2) The Examiner suggested that the normal SMN sequence be referenced with a SEQ ID NO: in the claims, so that there is a base sequence from which the deletions, truncations or mutations are being detected. The claims have been amended to recite that the a gene encodes a survival motor neuron protein of SEQ ID NO:22.

Meaning of "SSCP"

During the interview, the recitation of "SSCP" in the claims was discussed and the Examiner suggested that the term "SSCP" is indefinite. As noted by Applicants' representative during the Interview, "SSCP" analysis was well-known at the time of the invention and therefore one skilled in the art would understand the analysis encompassed by recitation of "SSCP analysis." As evidence that the term was well-known at the time of the invention, submitted herewith are three review articles that discuss the use of SSCP in mutational analysis of DNA. As such, Applicants believe that the term would have been readily recognized and understood by one practicing the invention in the mid-1990's.

Recitation of digestion with restriction enzymes

The claims that recite digestion of the gene with restriction enzymes have been further amended as requested by the Examiner to include a conclusion step.

Hybridization conditions

The Examiner questioned the support for the hybridization conditions recited, for example, in claim 53. During the interview, the Examiner indicated that the disclosure in Example 6 of the hybridization conditions is not by itself enough to support inserting those conditions into the claims directed to screening with probes.

Attached hereto in support of insertion of the hybridization conditions of Example 6 into the claims is an excerpt from Sambrook et al. (1989), pages 9.47-9.57. The excerpted section of Sambrook et al. pertains to the hybridization of probes to immobilized nucleic acids. In particular pages 9.56 and 9.57 pertain to the hybridization of probes to genomic DNA, which is relevant to the invention of claim 53. Specific points of concern regarding hybridizing nucleic acid probes to genomic DNA

are discussed in the attached excerpt from Sambrook et al. Importantly, nowhere does the relevant portion of Sambrook et al. discuss the use of particular hybridrization conditions as a point of concern with hybridizing probes to genomic DNA, suggesting that the conditions that are applicable to other applications, such as those in Example 6 of the specification, are also suitable for screening genomic DNA with a probe.

Recitation of SEQ ID NOS in the specification

Finally, the Examiner noted that there are some instances in the specification were SEQ ID NOS: need to be inserted. The specification has been reviewed and amended to insert reference to the Sequence Listing as appropriate.

Applicants believe the above amendments and remarks address the issues discussed during the interview. If the Examiner has any questions or would like to discuss the application further he is invited to please contact MaryAnne Armstrong, PhD (Reg. No. 40,069) at (703) 205-8000.

A marked-up version of the amended portions of the specification and claims showing all changes is attached.

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If necessary, the Commissioner is hereby authorized, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____

Marc S. Weiner, #32,181
MaryAnne Armstrong, Ph.D., #40,069

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

MSW/MAA/
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MARKED-UP VERSION SHOWING CHANGES

IN THE SPECIFICATION

The specification has been amended as follows.

The paragraph beginning at page 6, line 15, has been amended as follows.

--The inventors have identified two genes respectively designated T-BCD541 **(SEQ ID NO:12)** and C-BCD541 **(SEQ ID NO:10)**, which are involved in motor neuron diseases.--

The paragraph beginning at page 6, line 17, has been amended as follows.

--The T-BCD541 gene **(SEQ ID NO:12)** is responsible for the motor neuron diseases of the SMA type, since its alteration either by partial or total deletion, by mutation or any other modification, is sufficient to lead to a pathological state at the clinical electromyographic or muscle morphological levels.--

The paragraph beginning at page 6, line 21, has been amended as follows.

--The C-BCD541 gene **(SEQ ID NO:10)** is different from the T-BCD541 gene **(SEQ ID NO:12)**, at the level of the cDNA, since two

nucleotides are modified. This C-BCD541 gene is nevertheless not correctly processed during the transcription in controls and patients suffering from motor neuron diseases. The genomic DNA of the C-BCD541 gene is not correctly spliced during the transcription providing thus for an abnormal transcript. The difference between the splicing of the T-BCD541 and C-BCD541 gene results from differences in the sequence of the introns of these genes.--

The paragraph beginning at page 7, line 3, has been amended as follows.

--The present invention thus further characterizes the structure and organization of the human SMN gene which was found to be approximately 20 kb in length and consists of 9 exons interrupted by 8 introns. The nucleotide sequence, amino acid sequence as well as the exon-intron boundaries of the human SMN gene is set forth in Figure 10 **(SEQ ID NO:22)**. All exon-intron boundaries display the consensus sequence found in other human genes. A polyadenylation consensus site is localized about 550 bp downstream from the stop codon (Figure 10). The entire intron/exon structure of the SMN gene permits the

characterizations of the SMN gene mutations in SMA disease or other motor neuron diseases.--

The paragraph beginning at page 7, line 12, has been amended as follows.

--The present invention also defines means for the detection of genomic abnormalities relating to motor neuron diseases at the level of the T-BCD541 gene **(SEQ ID NO:12)** or at the level of the C-BCD541 gene **(SEQ ID NO:10)**.--

The paragraph beginning at page 9, line 18, has been amended as follows.

--In a particular embodiment, the invention relates also to a nucleotide sequence comprising nucleotides 34-915 of the sequence of Figure 3, **(SEQ ID NOS:12 and 13)**, or to a sequence comprising nucleotides 34 to 915 of the sequence of Figure 2 **(SEQ ID NOS:10 and 11)**.--

The paragraph beginning at page 9, line 21, has been amended as follows.

--These nucleotide sequences correspond to the coding sequence of respectively the T-BCD541 gene **(SEQ ID NO:12)** and C-BCD541 gene **(SEQ ID NO:10)**.--

The paragraph beginning at page 18, line 6, has been amended as follows.

--In another aspect, polyclonal rabbit antiserum were generated against synthetic peptides corresponding to the amino acid sequence of Figure 1 **(SEQ ID NO:9)**, 8 **(SEQ ID NO:19)** and 12 **(SEQ ID NO:10)**, including the amino acid terminus and the carboxy terminus.--

IN THE CLAIMS:

Claims 21, 30, 45, 52-54, 57, and 64-67 have been amended as follows.

21. (Three times amended) A kit for the *in vitro* detection of a truncation, a deletion or a mutation in ~~the~~ **a** survival motor neuron gene **encoding the amino acid sequence of SEQ ID NO:22,** comprising:

a ~~set~~ **pair** of primers wherein said primers **consist essentially of nucleic acid sequences selected from the group consisting of SEQ ID NOS:5-8 and 24-57** ~~are contained within the sequence of nucleotides 921 to 1469 of SEQ ID NO: 12 and are suitable for amplification of a fragment of said sequence~~;

reagents for amplifying DNA with said primers; and

a probe for the detection of the amplified product.

30. (Three times amended) A method for detecting the presence or absence of a truncation, a deletion or a mutation in **a survival motor neuron gene encoding the amino acid sequence of SEQ ID NO:22,** ~~the Survival Motor Neuron gene~~ in a DNA sample, **which comprises** ~~said method comprising~~:

(d) amplifying said DNA in the sample with primers, wherein said primers **consist essentially of nucleic acid sequences selected from the group consisting of SEQ ID NOS:5-8 and 24-57** ~~are contained in the sequence of nucleotides 921 to 1469 of SEQ ID NO: 12 and are suitable for amplification of a fragment of said sequence~~;

(e) subjecting said amplified DNA to a Single-Strand Conformation Polymorphism (SSCP) analysis, wherein the

analysis comprises comparing a pattern of DNA fragments obtained from the patient sample to a pattern of DNA fragments obtained from a control sample to detect alterations in the patient gene; and

(f) detecting the presence or absence of said truncation, deletion or mutation in the Survival Motor Neuron gene.

45. (Amended) The method of claim 44, wherein said determining includes

subjecting said exon 7 comprising nucleotide fragment to restriction enzyme digestion,

subjecting said exon 8 comprising nucleotide fragment to restriction enzyme digestion, and

analyzing enzymatic digestion products produced by said enzymatic digestions **by comparing the enzymatic digestion products from the biological sample to enzymatic digestion products of exon 7 or exon 8 of the survival motor neuron gene from normal tissue**,

wherein an alteration of either exon 7 or exon 8 with reference to normal tissue is evidenced by an altered restriction enzymatic digestion pattern in one or both of said exons.

52. (Amended) The method of claim 51, wherein said determining includes

subjecting said exon 7 comprising nucleotide fragment to restriction enzyme digestion,

subjecting said exon 8 comprising nucleotide fragment to restriction enzyme digestion, and

analyzing enzymatic digestion products produced by said enzymatic digestions **by comparing the enzymatic digestion products from the biological sample to enzymatic digestion products of exon 7 or exon 8 of the survival motor neuron gene from normal tissue**,

wherein an alteration of either exon 7 or exon 8 with reference to normal tissue is evidenced by an altered restriction enzymatic digestion pattern in one or both of said exons.

53. (Twice amended) A kit for the *in vitro* detection of a truncation, a deletion or a mutation in the Survival Motor Neuron gene **of SEQ ID NO:22**, wherein said kit comprises a probe which comprises at least 9 nucleotides within a sequence of SEQ ID NO: 12 or 13 or hybridizes with a sequence of SEQ ID NOS: 1,

2, or 10-13 under conditions having the stringency of 10% Dextran Sulphate Sodium, 1M NaCl, 0.05M Tris-HCl pH 7.5, 0.005M EDTA and 1% SDS at 65°C.

54. (Twice amended) A method of identifying the presence or absence of a mutation in the Survival Motor Neuron (SMN) gene **of SEQ ID NO:22** in a nucleic acid sample, comprising

(c) subjecting the nucleic acid in the sample to digestion by a restriction endonuclease, wherein restriction fragments resulting from said digestion of a mutated SMN gene differ from those obtained from a T-BCD541 gene of SEQ ID NO:~~22~~ **12**; and

(d) identifying the presence or absence of a mutation in the SMN gene in the subject.

57. (Once Amended) The method of claim 56, wherein said polymerase chain reaction is performed with a set of primers which are contained in the sequence comprising ~~nucleotides 921 to 1469 or SEQ ID NO:12~~, or which comprise a sequence selected from SEQ ID Nos: 5 to 8 and 24 to 57.

64. (Twice amended) A kit for the *in vitro* detection of a defect in the Survival Motor Neuron gene **of SEQ ID NO:22**, comprising:

a set of primers wherein said primers comprise a sequence selected from SEQ ID NOS: 5 to 8 and 24 to 57;

PCR reagents for amplifying DNA with said primers; and

a probe for the detection of the amplified product.

65. (Twice amended) A method for detecting the presence or absence of a truncation, a deletion or a mutation in the Survival Motor Neuron gene **of SEQ ID NO:22**, wherein the gene is present in a DNA sample obtained from an individual, said method comprising :

(a) amplifying said DNA with primers **consisting essentially of nucleic acid sequences** ~~, wherein said primers are~~ selected from the group of SEQ ID NOS: 5 to 8;

(b) subjecting said amplified DNA to a Single-Strand Conformation Polymorphism (SSCP) analysis, wherein the analysis comprises comparing a pattern of DNA fragments obtained from the patient DNA sample to a pattern of DNA fragments obtained from a control DNA sample; and

(c) detecting the presence or absence of said truncation, deletion or mutation in the Survival Motor Neuron gene.

66. (Amended) A method for detecting the presence or absence of a truncation, a deletion or a mutation in the Survival Motor Neuron gene **of SEQ ID NO:22**, wherein the gene is present in a DNA sample obtained from an individual, said method comprising :

(a) amplifying said DNA with primers **consisting essentially of nucleic acid sequences** ~~, wherein said primers are~~ selected from the group of SEQ ID NOS: 24 to 57;

(b) subjecting said amplified DNA to a Single-Strand Conformation Polymorphism (SSCP), wherein the analysis comprises comparing a pattern of DNA fragments obtained from the patient sample to a pattern of DNA fragments obtained from a control sample; and

(c) detecting the presence or absence of said truncation, deletion or mutation in the Survival Motor Neuron gene.

67. (Amended) A method for detecting the presence or absence of a truncation, a deletion or a mutation in the Survival Motor

Neuron gene **of SEQ ID NO:22**, wherein the gene is present in a DNA sample obtained from an individual, said method comprising :

(a) amplifying said DNA with primers, **consisting essentially of nucleic acid sequences** ~~, wherein said primers are~~ selected from the group of sequences which are inverted complementary sequences to SEQ ID NOS: 5 to 8;

(b) subjecting said amplified DNA to a Single-Strand Conformation Polymorphism (SSCP), wherein the analysis comprises comparing a pattern of DNA fragments obtained from the patient sample to a pattern of DNA fragments obtained from a control sample; and

(c) detecting the presence or absence of said truncation, deletion or mutation in the Survival Motor Neuron gene.